9. Guillot C, Badcock J, Clow K, Cram J, Dergousoff S, Dibernardo A, et al. Sentinel surveillance of Lyme disease risk in Canada, 2019: results from the first year of the Canadian Lyme Sentinel Network (CaLSeN). Can Commun Dis Rep. 2020;46:354–61. https://doi.org/10.14745/ccdr.v46i10a08
10. Bouchard C, Dibernardo A, Koffi J, Wood H, Leighton PA, Lindsay LRN. N Increased risk of tick-borne diseases with climate and environmental changes. Can Commun Dis Rep. 2019;45:83–9. https://doi.org/10.14745/ccdr.v45i04a02

Address for correspondence: David J. Haldane, Division of Microbiology, Department of Pathology and Laboratory Medicine Nova Scotia Health, 326A MacKenzie Building, 5788 University Ave, Halifax, NS B3H 1V8, Canada; email: david.haldane@nshealth.ca

Viral Zoonoses in Small Wild Mammals and Detection of Hantavirus, Spain

Silvia Herrero-Cófreces, François Mougeot, Tarja Sironen, Hermann Meyer, Ruth Rodríguez-Pastor, Juan José Luque-Larena

Author affiliations: Universidad de Valladolid, Palencia, Spain (S. Herrero-Cófreces, R. Rodríguez-Pastor, J.J. Luque-Larena); Instituto de Investigación en Recursos Cinegéticos, Ciudad Real, Spain (F. Mougeot); University of Helsinki, Helsinki, Finland (T. Sironen); Bundeswehr Institute of Microbiology, Munich, Germany (H. Meyer)

DOI: https://doi.org/10.3201/eid2806.212508

We screened 526 wild small mammals for zoonotic viruses in northwest Spain and found hantavirus in common voles (*Microtus arvalis*) (1.5%) and high prevalence (48%) of orthopoxvirus among western Mediterranean mice (*Mus spretus*). We also detected arenavirus among small mammals. These findings suggest novel risks for viral transmission in the region.

Wildlife viromes harbor potentially threatening zoonoses for humans that require increased effort in identification and surveillance (*1*). Rodents are considered main reservoirs of emerging zoonoses (*2*), and the large population fluctuations of reservoir species play a key role in modulating infection risk (*3*). Anthropogenic land-use changes, agricultural intensification, and irrigation also favor rodent invasions and risk for pathogen spillover (*4*). The common vole (*Microtus arvalis*) is a widespread rodent inhabiting intensified farming landscapes in northwestern Spain, where population numbers and pathogen prevalence lead to spillover of zoonotic bacteria such as *Francisella tularensis* and *Bartonella* spp. (*5*).

We report the prevalence of rodent-borne zoonotic viruses in Europe (i.e., hantavirus, arenavirus [lymphocytic choriomeningitis virus (LCMV)], and orthopoxvirus) (*6*) among the small mammals inhabiting farming landscapes. We also report the effect of natural fluctuations of common vole numbers on viral prevalence (phase dependence). Our study was conducted in intensively farmed landscapes, in the Tierra de Campos region of Castilla-y-León, northwestern Spain (*7*), where the small mammal population is mainly composed of 4 species: common vole, long-tailed field mouse (*Apodemus sylvaticus*), western Mediterranean mouse (*Mus spretus*), and greater white-toothed shrew (*Crocidura russula*) (*7*).

We live-trapped small mammals during March 2013–March 2019. We collected samples from blood, spleen, liver, and lungs by using standard protocols and stored them at –23°C until molecular analysis could be performed (Appendix, https://wwwnc.cdc.gov/EID/article/28/6/21-2508-App1.pdf). We owned all necessary licenses and permits for conducting this study.

We detected specific hantavirus, LCMV, and orthopoxvirus IgG in serum samples by using immunofluorescence assay. We used fluorescein isothiocyanate (FITC) anti-IgG as a secondary antibody and evaluated all slides under a fluorescence microscope. For molecular analysis, we isolated RNA from liver and lung tissues and DNA from a mix of liver and spleen. We performed single-step reverse transcription PCR (RT-PCR) for LCMV detection in the liver, nested reverse transcription PCR for hantavirus detection in lung samples, and conventional pan-poxvirus PCR method followed by an additional orthopoxvirus-specific PCR for orthopoxvirus detection in the mix samples. We used generalized linear models to test variations of prevalence between species and calculate prevalence in common voles according to host sex (male or female), trapping month (March, July, or November), and population density phase (increase, peak, or crash).

We screened 526 individual animals from 4 species for the presence of 3 viruses (Table; Appendix). We found evidence of hantavirus infection only in

Table. Prevalence of hantavirus, arenavirus (LCMV), and orthopoxvirus in 4 small mammal species from the Tierra de Campos region, Castilla-y-León, northwest Spain, 2013–2019*

Species	Common name	Virus	Prevalence		
			Screening method	No. positive/screened	% Positive (95% CI)
Apodemus sylvaticus	Long-tailed field mouse	LCMV	IFA	2/34	5.9 (0.7–19.7)
			PCR	0/2	Not tested
		Hantavirus	IFA	0/34	Not tested
			PCR	Not tested	Not tested
		Orthopoxvirus	IFA	0/34	Not tested
			PCR	Not tested	Not tested
Crocidura russula	Greater white-toothed shrew	LCMV	IFA	0/7	Not tested
			PCR	1/9	11.1 (0.3–48.2)
		Hantavirus	IFA	0/7	Not tested
			PCR	0/9	Not tested
		Orthopoxvirus	IFA	0/7	Not tested
			PCR	Not tested	Not tested
Microtus arvalis	Common vole	LCMV	IFA	8/382	2.1 (0.9–4.1)
			PCR	2/89	2.2 (0.3–7.9)
		Hantavirus	IFA	3/382	0.8 (0.2–2.3)
			PCR	4/62	6.5 (1.8–15.7)
		Orthopoxvirus	IFA	5/382	1.3 (0.4–3.0)
			PCR	0/243	Not tested
Mus spretus	Western Mediterranean mouse	LCMV	IFA	0/25	Not tested
			PCR	Not tested	Not tested
		Hantavirus	IFA	0/25	Not tested
			PCR	Not tested	Not tested
		Orthopoxvirus	IFA	12/25	48.0 (27.8–68.7)
			PCR	Not tested	Not tested
All hosts		LCMV	All tests	13/526	2.5 (1.3–4.2)
		Hantavirus	All tests	7/458	1.5 (0.6–3.1)
		Orthopoxvirus	All tests	17/510	3.3 (2.0–5.3)

*LCMV, lymphocytic choriomeningitis virus.

common voles, at an average prevalence of 1.6% (95% CI 0.6%–3.3%; 7/438). Positive results for LCMV infection (either by immunofluorescence assay or PCR) were detected in 5.9% (95% CI 0.7%–19.7%) of long-tailed field mice (2/34, 11.1% (95% CI 0.7%–48.2%) of shrews (1/9), and 2.2% (95% CI 1.1%–4.0%) of common voles (10/458). Orthopoxvirus IgG was present in 1.3% (95% CI 0.4%–3.0%) of common voles (5/382) and in 48% (95% CI 27.8%–68.7%) of western Mediterranean mice (12/25), and we observed significant differences between both species ($\chi^2 = 59.643$, d.f. = 3; $p < 0.001$). In long-tailed field mice, we only detected LCMV during summer (July). In common voles, we found no effect of cycle phase or month on virus prevalence (Appendix), but LCMV prevalence differed between sexes ($\chi^2 = 5.189$, d.f. = 1; $p = 0.023$) and was higher in males (3.7%; 95% CI 1.6%–7.1%) than in females (0.8%; 95% CI 0.1%–0.3%).

Recent surveys of viral zoonoses in Spain have shown low antibody prevalence of LCMV (1.7%) (*8*) and hantavirus (0.06%) (*9*) among humans. Hantavirus antibodies were detected in red foxes (*Vulpes vulpes*) (*10*), and LCMV antibodies were detected in long-tailed field mice and red foxes (*8*,*10*). Our study detected hantavirus in a wild rodent reservoir in Spain. The reported prevalence was low (1.6%) and did not differ between the phases of the common vole population cycle. However, the cyclic dynamic of this rodent host, which harbored all 3 virus species screened, may influence the risks associated with contact with infected rodents. Common voles can reach densities of up to 1,000 per hectare during population peaks, so the infected proportion may become a considerable public health concern. Orthopoxvirus infection risk is of growing concern in Europe because of the absence of smallpox vaccination among the human population <45 years of age (*6*). Because half of all the western Mediterranean mice analyzed were positive for orthopoxvirus, the potential transmission risk for the virus from this rodent to humans should be considered and further confirmed with larger sample sizes.

Further investigation is required regarding the molecular nature and infectivity of the hantavirus and orthopoxvirus detected, as well as their circulation pathways, which will help to uncover possible transmission routes and determine more precisely the level of infection risk to human populations. Our results can be used by local authorities to refine virus surveillance, including clinical diagnosis of new viruses, and improve public health strategies to prevent and minimize zoonotic risks for persons living in areas recurrently affected by outbreaks linked to common voles.

Acknowledgments

We thank Hussein Alburkat for helping with immunologic assays and the Instituto de Salud Carlos III for collaborating with DNA extraction.

This work was funded by the projects ECOTULA (grant no. CGL2015-66962-C2-1-R) and BOOMRAT (grant no. PID2019-109327RB-I00) funded by the Government of Spain, and GESINTTOP, co-funded by Instituto Tecnológico Agrario de Castilla-y-León–Junta de Castilla-y-León, Diputación Provincial de Palencia, and Diputación Provincial de Valladolid. S.H.C. was supported by a Ph.D. studentship from Junta de Castilla-y-León (co-funded by the European Social Fund) and an Erasmus+ Mobility grant. R.R.P. was supported by a PhD studentship from the University of Valladolid (co-funded by Banco Santander).

About the Author

Ms. Herrero-Cófreces is a biologist and doctoral student in the Department of Agroforestry Sciences at the University of Valladolid in Palencia, Spain. Her primary research interests include rodents, rodent-borne diseases, and ecology of zoonoses.

References

1. Carroll D, Daszak P, Wolfe ND, Gao GF, Morel CM, Morzaria S, et al. The global virome project. Science. 2018;359:872–4. https://doi.org/10.1126/science.aap7463
2. Han BA, Schmidt JP, Bowden SE, Drake JM. Rodent reservoirs of future zoonotic diseases. Proc Natl Acad Sci U S A. 2015;112:7039–44. https://doi.org/10.1073/pnas.1501598112
3. Cavanagh RD, Lambin X, Ergon T, Bennett M, Graham IM, van Soolingen D, et al. Disease dynamics in cyclic populations of field voles (*Microtus agrestis*): cowpox virus and vole tuberculosis (*Mycobacterium microti*). Proc R Soc London Ser B Biol Sci. 2004;271:859–67.
4. Faust CL, McCallum HI, Bloomfield LSP, Gottdenker NL, Gillespie TR, Torney CJ, et al. Pathogen spillover during land conversion. Ecol Lett. 2018;21:471–83. https://doi.org/10.1111/ele.12904
5. Herrero-Cófreces S, Mougeot F, Lambin X, Luque-Larena JJ. Linking zoonosis emergence to farmland invasion by fluctuating herbivores: common vole populations and tularemia outbreaks in NW Spain. Front Vet Sci. 2021;8:698454. https://doi.org/10.3389/fvets.2021.698454
6. Kallio-Kokko H, Uzcategui N, Vapalahti O, Vaheri A. Viral zoonoses in Europe. FEMS Microbiol Rev. 2005;29:1051–77. https://doi.org/10.1016/j.femsre.2005.04.012
7. Rodríguez-Pastor R, Luque-Larena JJ, Lambin X, Mougeot F. "Living on the edge": the role of field margins for common vole (*Microtus arvalis*) populations in recently colonised Mediterranean farmland. Agric Ecosyst Environ. 2016;231:206–17. https://doi.org/10.1016/j.agee.2016.06.041
8. Lledó L, Gegúndez MI, Saz JV, Bahamontes N, Beltrán M. Lymphocytic choriomeningitis virus infection in a province of Spain: analysis of sera from the general population and wild rodents. J Med Virol. 2003;70:273–5. https://doi.org/10.1002/jmv.10389
9. Lledó L, Klingström J, Gegúndez MI, Plyusnina A, Vapalahti O, Saz JV, et al. Hantavirus infections in Spain: analysis of sera from the general population and from patients with pneumonia, renal disease and hepatitis. J Clin Virol. 2003;27:296–307. https://doi.org/10.1016/S1386-6532(02)00228-7
10. Lledó L, Serrano JL, Giménez-Pardo C, Gegúndez I. Wild red foxes (*Vulpes vulpes*) as sentinels of rodent-borne hantavirus and lymphocytic choriomeningitis virus in the province of Soria, northern Spain. J Wildl Dis. 2020;56:658–61. https://doi.org/10.7589/2019-09-239

Address for correspondence: Silvia Herrero-Cófreces, Departamento de Ciencias Agroforestales, Escuela Técnica Superior de Ingenierías Agrarias, Universidad de Valladolid, Avenida de Madrid 50, Palencia, E-34004, Spain; email: silvia.herrero.cofreces@uva.es

Detecting SARS-CoV-2 Omicron B.1.1.529 Variant in Wastewater Samples by Using Nanopore Sequencing

Lasse D. Rasmussen, Stine R. Richter, Sofie E. Midgley, Kristina T. Franck

Author affiliation: Statens Serum Institut, Copenhagen, Denmark

DOI: https://doi.org/10.3201/eid2806.220194

We report wastewater surveillance for SARS-CoV-2 variants of concern by using mutation-specific, real-time PCR and rapid nanopore sequencing. This surveillance might be useful for an early warning in a scenario in which a new variant is emerging, even in areas that have low virus incidences.

To limit spread of novel SARS-CoV-2 variants such as Omicron B.1.1.529, early detection is crucial. Wastewater surveillance has been suggested as an early warning system for SARS-CoV-2 spread in low-prevalence areas or communities where human testing is limited (*1*).

We provide a method to rapidly determine the presence of Omicron in wastewater samples that have low viral load, in which the Omicron genome

Article DOI: https://doi.org/10.3201/eid2806.212508

Viral Zoonoses in Small Wild Mammals and Detection of Hantavirus, Spain

Appendix

Study Site and Small Mammal Guild

The study was conducted in the "Tierra de Campos" region, Castilla-y-León, NW Spain, in farming landscapes dominated by cereal fields with scattered irrigated crops (mainly alfalfa) and interspersed by fallows and remnant semi-natural vegetation (*1*). Climate is continental-Mediterranean, characterized by wide seasonal temperature oscillations: long winters with frequent frost events, hot summers with drought periods, and precipitation concentrated during spring and autumn (*2*).

In the studied habitats, more than 95% of the small mammal community belongs to a guild composed of three rodents and one insectivore: common voles (*Microtus arvalis*), long-tailed field mice (*Apodemus sylvaticus*), western Mediterranean mice (*Mus spretus*), and great white-toothed shrews (*Crocidura russula*) (*1*). The common vole is a fossorial rodent (*3*) characterized by population peaks recorded every 3 years in the study area (*4*), whereas mouse species show seasonal fluctuations (*5*,*6*). The great white-toothed shrew is the main insectivore species in the guild (*7*).

Trapping Design and Sample Collection

Fieldwork consisted of periodic live-trapping events three times per year (March, July, November) from March 2013 to March 2019. Our sampling design was spatially stratified, monitoring randomly selected fields from those available among the three most relevant crops (i.e., cereals, alfalfa and fallows). We used Sherman© traps (8 cm × 9 cm × 23 cm; LFAHD Sherman©) baited with carrot and apple, set them open in the morning and checked them 24 h later. See (*1*) and (*4*) for more details. Each trapped animal was provided with a unique code; date, site and crop field were recorded.

Once in the laboratory, animals were euthanized with CO_2, following a humane protocol approved by the ethics committee of our institution (CEEBA, Universidad de Valladolid; authorization code: 4801646). Immediately after death, animals were weighed, sexed and blood was collected by cardiac puncture using a 25G needle with a sterile 1.5 mL syringe and coated with heparin. Samples were centrifuged at 3000 rpm for 20 min. Serum was collected and stored at −23°C. Animals trapped from 2013 to 2018 were stored at −23°C until dissection; however, animals trapped in 2019, were dissected immediately following euthanasia. Following standard protocols, the spleen, liver, and lungs were individually weighted (±0.005 g) and stored at −23°C until molecular analysis could be performed, except for animals trapped in 2019, which were frozen in Invitrogen® RNAlater® Stabilization Solution (Thermo Fisher Scientific Inc., Waltham, MA, USA). We screened 526 individuals from four species of the small mammal guild for the presence of three viruses (https://wwwnc.cdc.gov/EID/article/28/6/21-2508-T1.htm; Appendix Tables 1 and 2).

Viruses Screened: Hantavirus, Arenavirus and Orthopoxvirus

Hantaviruses are RNA rodent-borne viruses belonging to the family Bunyaviridae transmitted by aerosols from rodent excreta. They cause persistent infection in hosts. In Europe, there are five different hantaviruses: Tula virus, carried by *M. arvalis*; Puumala virus, by bank voles (*Myodes glareolus*); Dobrava virus, by yellow-necked field mice (*A. flavicollis*); Saarema virus, by striped field mouse (*A. agrarius*); and Seoul virus, by rats (*Rattus norvegicus*, *R. rattus*) (*8*,*9*). Arenaviruses are RNA viruses belonging to the family Arenaviridae, possibly transmitted by rodent fluids. They cause chronic infection in hosts. Lymphocytic choriomeningitis virus (LCMV) is the only arenavirus endemic in Europe. The house mouse (*M. musculus*) is the main reservoir and carrier of this virus although it can circulate in other rodent species (*8*,*10*). Orthopoxviruses are DNA viruses that can be directly transmitted by rodents and other hosts such as cats. Cowpox virus is the main virus of this type in Europe and causes a rapidly recovering infection in hosts. Some voles (*Myodes* spp., *Microtus* spp.) and mice (*Apodemus* spp.) are the main reservoirs (*8*,*11*).

Serologic Screening

Specific IgG antibodies were detected from specimens with serum samples available using an IFA following Forbes et al. (*12*). Hantavirus-, LCMV-, or orthopoxvirus-infected Vero cells mixed with uninfected cells were spotted and fixed on the IFA slide. The slides were stored at −70 ◦C until use.

The samples were diluted 1:20 in phosphate-buffered saline solution (PBS) and tested for hantavirus virus, LCMV, and orthopoxvirus. Seropositive human serum was used as a positive control for hantavirus and orthopoxvirus-IFA tests, while positive mouse serum was the positive control for LCMV-IFA. PBS was used as a negative control in all cases. Slides were warmed up to room temperature and 25 µL of each diluted sample, positive and negative controls were added in independent wells. The slides were incubated at +37°C in a moist chamber for 30 min, followed by three washing steps in PBS for 5 min each and one last washing step with Milli-Q water. After slides were dried under a fun, a 25 µL amount of secondary antibody was used in every well. Fluorescein (FITC) AffiniPure Fab Fragment Goat Anti-Mouse IgG (H+L) (Jackson InmunoResearch, Ely, UK), diluted 1:30, was used as secondary antibody in animal sample, negative control and LCMV positive control wells. Fluorescein (FITC) AffiniPure $F(ab')_2$ Fragment Goat Anti-Human IgG (H+L) (Jackson InmunoResearch, Ely, UK), diluted 1:100, was used in hantavirus and orthopoxvirus positive control wells. After incubation at +37°C in a moist chamber for 30 min, slides were washed three times in PBS for 5 min each time and one last washing step with Milli-Q water. When completely dry, coverslips were placed on the slides with a mounting medium, and slides were evaluated under a fluorescence microscope. Slides were protected from light and stored at +4°C. Samples with unclear results were repeated, diluting the original sample 1:10, 1:40 and 1:80.

Molecular Detection

Small mammals with positive results to hantavirus or LCMV, and those with undetermined results (i.e., common voles with no serum available and all the shrews), were selected for PCR analyses focused on RNA viruses' detection. RNA was isolated by using Invitrogen® TRIzol® Reagent (Thermo Fisher Scientific Inc., Waltham, MA, USA), following

the manufacturer's instructions. RNA concentration was measured with a Nanodrop® ND-1000 spectrophotometer.

For LCMV, a total of 100 liver samples (10 IFA-positives, 5 common voles with unclear IFA results, 76 common voles with no sera available and 9 shrews) were tested. One-step reverse transcription PCR (RT-PCR) was performed for arenavirus detection, using Old-World Arena Rivigene primers and Invitrogen® one-step RT-PCR kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). RT-PCR reaction was performed using Thermal cycler. The cycling protocol consisted of six steps: reverse transcription step 30 min at 50°C; followed by one temp step of 2 min at 94°C; 45 cycles of 20 s at 94°C; one 30-second step at 55°C; one 60-second step at 72°C; and a final step of 5 min at 72°C. In each PCR run, positive and negative controls were included. Finally, PCR products were run on agarose gel and visualized under UV light. See (*13*) for more details.

For hantavirus, we checked 71 lung samples (3 IFA-positives, 3 common voles with unclear IFA result, 56 common voles with no sera available and 9 shrews). RT reaction mixture was prepared using RevertAid Premium Reverse transcription (Thermo Fisher Scientific Inc., Waltham, MA, USA) and a previous phase of RNA amplification was performed as follows: 30 min at 55°C and 5 min at 85°C. Nested RT-PCR was done for hantavirus detection, using Phusion Flash High-Fidelity PCR Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA). The first PCR to obtain cDNA was carried out as follows: an initial step of 10 s at 98°C; 7 cycles of 1 s at 98°C, followed by 5 s at 50°C and 15 s at 72°C; 35 cycles of 1 s at 98°C, followed by 5 s at 55°C and 15 s at 72°C; and a last step of 1 min at 72°C. The second PCR to obtain DNA comprised an initial step of 10 s at 98°C; 40 cycles of 1 s at 98°C, followed by 5 s at 60°C and 15 s at 72°C; and a last step of 1 min at 72°C. The second round PCR products were visualized in agarose gels and visualized under UV light. See (*14*) for more details.

A total of 243 common voles sampled from March 2013 to March 2015 (62 animals with no sera available) were tested for orthopoxvirus DNA (using a mix of liver and spleen). DNA was isolated with QIAamp DNA Mini Kit® (QIAGEN, Valencia, CA, USA) and measured with Nanodrop ND-1000. The pan-poxvirus PCR method (*15*) and a real-time PCR using the RealStar® Orthopoxvirus PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) were used. This kit includes three independent PCRs: (i) an internal control to detect and avoid problems

with inhibition, (ii) a PCR that specifically detects variola virus and (iii) an OPV-specific PCR, respectively (*16*). The reaction mix was set up according to the manufacturer's instructions.

Statistical Analysis

We obtained information on the prevalence of each host species. Prevalence data were summarized as the proportion of infected hosts (the number of infected hosts among hosts examined) and 95% confidence intervals (CI). We used generalized linear models (GLM) to test variations of prevalence between species, and prevalence in host species according to host sex (male, female), trapping month (March, July, November) and population density phase (increase, peak, crash) (Appendix Table 1). Because of sample size limitations, the prevalence model was calculated only for common voles (Appendix Table 2). A p-value of <0.05 was considered significant. We used the "lme4" package (*17*) of the R software version 3.6.1 (https://cran.r-project.org/bin/windows/base/old/3.6.1).

References

1. Rodríguez-Pastor R, Luque-Larena JJ, Lambin X, Mougeot F. "Living on the edge": the role of field margins for common vole (*Microtus arvalis*) populations in recently colonised Mediterranean farmland. Agric Ecosyst Environ. 2016;231:206–17. https://doi.org/10.1016/j.agee.2016.06.041

2. Rivas-Martínez S, Penas Á, del Río S, Díaz González TE, Rivas-Sáenz S. Bioclimatology of the Iberian Peninsula and the Balearic Islands. In: Loidi J, editor. The vegetation of the Iberian Peninsula. Cham (Switzerland): Springer International Publishing; 2017. p. 29–80.

3. Hernández MC, Sánchez-González B, Navarro-Castilla Á, Barja I. Topillo campesino—*Microtus arvalis* (Pallas, 1779). Enciclopedia virtual de los vertebrados españoles. 2017 Mar 14 [cited 2021 Oct 20]. https://digital.csic.es/bitstream/10261/146730/4/micarv_v1.pdf

4. Mougeot F, Lambin X, Rodríguez-Pastor R, Romairone J, Luque-Larena JJ. Numerical response of a mammalian specialist predator to multiple prey dynamics in Mediterranean farmlands. Ecology. 2019;100:e02776. PubMed https://doi.org/10.1002/ecy.2776

5. Torre I, Arrizabalaga A, Díaz M. Ratón de campo (*Apodemus sylvaticus*. Linnaeus, 1758). Galemys. 2002;14:1–26.

6. Palomo LJ, Justo ER, Vargas JM. *Mus spretus* (Rodentia: Muridae). Mamm Species. 2009;840:1–10. https://doi.org/10.1644/840.1

7. Palomo LJ, Gisbert J, Blanco JC. Atlas y libro rojo de los mamíferos terrestres de España. Madrid: Dirección General para la Biodiversidad; 2007. p. 588.

8. Kallio-Kokko H, Uzcategui N, Vapalahti O, Vaheri A. Viral zoonoses in Europe. FEMS Microbiol Rev. 2005;29:1051–77. PubMed https://doi.org/10.1016/j.femsre.2005.04.012

9. Vapalahti O, Mustonen J, Lundkvist A, Henttonen H, Plyusnin A, Vaheri A. Hantavirus infections in Europe. Lancet Infect Dis. 2003;3:653–61. PubMed https://doi.org/10.1016/S1473-3099(03)00774-6

10. Meerburg BG, Singleton GR, Kijlstra A. Rodent-borne diseases and their risks for public health. Crit Rev Microbiol. 2009;35:221–70. PubMed https://doi.org/10.1080/10408410902989837

11. Kinnunen PM, Henttonen H, Hoffmann B, Kallio ER, Korthase C, Laakkonen J, et al. Orthopox virus infections in Eurasian wild rodents. Vector Borne Zoonotic Dis. 2011;11:1133–40. PubMed https://doi.org/10.1089/vbz.2010.0170

12. Forbes KM, Voutilainen L, Jääskeläinen A, Sironen T, Kinnunen PM, Stuart P, et al. Serological survey of rodent-borne viruses in Finnish field voles. Vector Borne Zoonotic Dis. 2014;14:278–83. PubMed https://doi.org/10.1089/vbz.2013.1526

13. Vieth S, Drosten C, Lenz O, Vincent M, Omilabu S, Hass M, et al. RT-PCR assay for detection of Lassa virus and related Old World arenaviruses targeting the L gene. Trans R Soc Trop Med Hyg. 2007;101:1253–64. PubMed https://doi.org/10.1016/j.trstmh.2005.03.018

14. Klempa B, Fichet-Calvet E, Lecompte E, Auste B, Aniskin V, Meisel H, et al. Hantavirus in African wood mouse, Guinea. Emerg Infect Dis. 2006;12:838–40. PubMed https://doi.org/10.3201/eid1205.051487

15. Li Y, Meyer H, Zhao H, Damon IK. GC content-based pan-pox universal PCR assays for poxvirus detection. J Clin Microbiol. 2010;48:268–76. PubMed https://doi.org/10.1128/JCM.01697-09

16. Olson VA, Laue T, Laker MT, Babkin IV, Drosten C, Shchelkunov SN, et al. Real-time PCR system for detection of orthopoxviruses and simultaneous identification of smallpox virus. J Clin Microbiol. 2004;42:1940–6. PubMed https://doi.org/10.1128/JCM.42.5.1940-1946.2004

17. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Softw. 2015;67.

Appendix Table 1. Number of samples analyzed from each trapping session in the "Tierra de Campos" region, Castilla-y-León, NW Spain, 2013–2019

			Host*(sample size)				
Year	Month	Phase	AS	CR	MA	MS	All species
2013	All months	Increase	10	7	51	10	78
	March		0	1	4	0	5
	July		5	6	15	0	26
	November		5	0	32	10	47
2014	All months	Peak	8	2	183	10	203
	March		3	1	63	6	73
	July		2	0	101	1	104
	November		3	1	19	3	26
2015	All months	Crash	2	0	15	0	17
	March		2	0	8	0	10
	July		0	0	0	0	0
	November		0	0	7	0	7
2016	All months	Peak	0	0	63	0	63
	March		0	0	3	0	3
	July		0	0	30	0	30
	November		0	0	30	0	30
2017	All months	Crash	0	0	61	0	61
	March		0	0	30	0	30
	July		0	0	30	0	30
	November		0	0	1	0	1
2018	All months	Increase	0	0	4	0	4
	March		0	0	1	0	1
	July		0	0	3	0	3
	November		0	0	0	0	0
2019	All months	Peak	14	0	81	5	100
	March		14	0	81	5	100
All years	All months		34	9	458	25	526

*AS, *Apodemus sylvaticus*; CR, *Crocidura russula*; MA, *Microtus arvalis*; MS, *Mus spretus*

Appendix Table 2. Results of generalized linear models (GLM) to test variations of prevalence in *Microtus arvalis* from "Tierra de Campos" region, Castilla-y-León, NW Spain, 2013–2019

Predictor	LCVM	Hantavirus	Orthopoxvirus
Sex	X^2 = 5189; df = 1, p = 0023	X^2 = 0204; df = 1, p = 0651	X^2 = 0292; df = 1, p = 0589
Month	X^2 = 3354; df = 2, p = 0187	X^2 = 0046; df = 2, p = 0977	X^2 = 1265; df = 2, p = 0531
Phase	X^2 = 4200; df = 2, p = 0122	X^2 = 2054; df = 2, p = 0358	X^2 = 2449; df = 2, p = 0294